AMENDMENTS

In the claims

Please amend claims 50, 51, 53, 55-57, 60, 68, 69, 72, 74, 75, 82, 84-86, 88, 89, 91, 92, 118, 119, and 159 as follows:

50. (Amended) A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating an AAV producer cell under conditions that are permissive for replication of AAV; said producer cell comprising: (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

b) lysing the producer cell after the incubation of step a) to produce an AAV producer cell lysate;

c) chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin; and

d) purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.

51. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said purifying step d) comprises subjecting the fractions to cation exchange chromatography.

53. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said rAAV vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

55. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the helper virus is introduced into the producer cell already introduced with the AAV packaging gene(s) and the rAAV vector.

56. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the rAAV vector and the helper virus are introduced simultaneously or sequentially into the producer cell already introduced with the AAV packaging gene(s).

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57. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV packaging gene(s) and the rAAV vector are introduced simultaneously or sequentially into the producer cell already introduced with the helper virus.

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60. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein at least one AAV split-packaging gene is introduced into the producer cell.

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68. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells of step a) are concentrated prior to lysis.

69. (Amended) A method of generating a population of rAAV particles according to claim 68, wherein the AAV producer cells of step a) are concentrated by centrifugation or by tangential flow filtration prior to lysis.

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72. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step b) is treated with a nuclease prior to chromatography.

74. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step b) is clarified prior to chromatography.

B7 75. (Amended) A method of generating a population of rAAV particles according to claim 74, wherein the AAV producer cell lysate of step b) is clarified by filtration or centrifugation prior to chromatography.

B8 82. (Amended) A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is selected from the group consisting of a heparin sulfate (HS) resin, a sulfopropyl (SP) resin, and a carboxymethyl (CM) resin.

84. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is conducted in a vessel selected from the group consisting of a tissue culture flask, a roller bottle, a spinner flask, a tank reactor, a fermentor, and a bioreactor.

85. (Amended) A method of generating a population of rAAV particles according to B9 claim 50, wherein said step a) of incubating the producer cell is conducted using a microcarrier.

86. (Amended) A method of generating a population of rAAV particles according to claim 84, wherein said bioreactor is a hollow-fiber, packed-bed or fluidized-bed bioreactor.

B10 cont'd. 88. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is conducted in a vessel selected from the group consisting of a spinner flask, a tank reactor and an air lift fermentor.

89. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is performed in rAAV medium essentially as shown in Table 2.

91. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein step a) is conducted for at least 5 days.

92. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein step a) of incubating the producer cell is conducted in a multi-liter bioreactor and wherein at least about 10^9 replicative units of rAAV per liter of bioreactor volume are isolated after step d).

118. (Amended) A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating an AAV producer cell under conditions that are permissive for replication of AAV and which comprise inducing a sub-lethal stress in the AAV producer cell; wherein said AAV producer cell comprising (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

b) lysing the producer cell after the incubation of step a) to produce an AAV producer cell lysate; and

c) purifying the AAV producer cell lysate to generate a population of recombinant adeno-associated virus (rAAV) particles, wherein said purifying step comprises chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin followed by purifying on either a cation exchange resin or by tangential flow filtration to generate a purified population of rAAV vector particles.

119. (Amended) The method of claim 118, wherein said purifying step c) comprises chromatographing the AAV producer cell lysate of step b) on at least one negatively -charged cation exchange resin followed by purifying on an anion exchange resin.

159. (Amended) A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating a producer cell in a cell culture medium under conditions comprising a condition that promotes release of rAAV particles, whereby rAAV particles are released from the producer cell into the culture medium, wherein the producer cell comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) helper virus function for AAV;

(b) harvesting the rAAV particles from the cell culture medium, thereby obtaining a population of rAAV particles; and

c) chromatographing the rAAV producer cell culture medium on a positively-charged anion exchange resin; and

d) purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.